Validation of a Whole Blood Serology Test for Diagnosing Helicobacter pylori in Chinese Patients

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Abstract

VALIDATION OF A WHOLE BLOOD SEROLOGY TEST FOR DIAGNOSING *HELICOBACTER PYLORI* IN CHINESE PATIENTS.

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Purpose To assess the accuracy of the FlexPack HP (Abbott Laboratory), a rapid whole blood immuno-chromatographic serology test, for diagnosing *Helicobacter pylori* infection in Hong Kong Chinese patients.

Methods Consecutive patients with dyspepsia presenting for endoscopic examination were recruited for FlexPack HP testing. Those having received previous anti-*H. pylori* therapy or those currently taking antibiotics or acid-suppressive drugs were excluded. In addition to having the FlexPack HP test, all patients were tested for *H. pylori* using histology, rapid urease test and a [^{13}C] urea breath test. Patients were considered to be infected with *H. pylori* when two or more of these three reference tests were positive.

Results One hundred patients were studied. The average age was 51 +/- 15 years (range 21 – 90 years), 55% of whom were female. Endoscopy revealed gastric ulcers in 5%, duodenal ulcers in 6%, gastric erosions in 7%, gastric erythema in 20% and no apparent disease in 62%. Fifty-four (54%) of the patients had *H. pylori* infection based on study definitions. FlexPack HP test characteristics were: sensitivity 82% (95% CI: 73 - 88%), specificity 85% (95% CI: 74 - 92%), positive predictive value 86% (95% CI: 77 - 93%) and negative predictive value 80% (95% CI: 70 - 86%). A faint line, interpreted as positive, was seen in 15 (15%) of the FlexPack HP results. No invalid tests occurred as assessed by the control panel integral to the FlexPack HP test.

Conclusion FlexPack HP was quick, convenient and accurate when used in a secondary care setting in Hong Kong Chinese patients. This test may be useful for in-office *H. pylori* testing.

Table of Contents

Introduction

History

Our understanding of *Helicobacter pylori* and its role in peptic ulcer disease has increased dramatically since its re-discovery in 1982 by Marshall and Warren who isolated the bacterium from gastric biopsy specimens taken from patients with gastritis (1). At that time, the micro-aerophilic, curved, spiral-shaped bacillus was named *Campylobacter pyloridis* since it resembled other *Campylobacters* both morphologically and in guanine/cytosine DNA content; later the name became *Campylobacter pylori* and finally *Helicobacter pylori* (2, 3). This urease-producing organism exists in close association with the epithelium of the gastric lumen. By 1984, Marshall and Warren had presented and published this finding along with their discovery of the association of *H. pylori* infection with both gastritis and peptic ulcer disease (4). Marshall even went so far as to ingest the live organism purposely to demonstrate the acute effects of infection in an effort to fulfill Koch's postulates (5).

Pathogenicity

The role of *H. pylori* as an important human pathogen became increasingly defined over the following years. *H. pylori* is associated with chronic gastritis (6), and gastric and duodenal ulcers (7, 8). More questionable studies have associated *H. pylori* with gastric adenocarcinoma (9, 10) and gastric lymphoma (11, 12). *H. pylori* might contribute to non-ulcer dyspepsia (13), migraine headaches (14), hyperemesis gravidarum (15, 16), ischemic heart disease (17) or even childhood growth retardation (18), although dubious and conflicting studies exist in some cases (19). In 1994 *H. pylori* was given

grade I (i.e., definite) carcinogen status by the International Agency for Research on Cancer (20).

Virulence

Virulence factors have been identified for *H. pylori* which may determine the likelihood of developing disease and influence its severity. The two main virulence factors identified to date are the toxins produced by the genes *vacA* (vacuolating cytotoxin gene A) and *cagA* (cytotoxin associated gene A). Both *vacA*+ and *cagA*+ strains are isolated more often from patients with peptic ulcers than from those without ulcerative disease. The *cagA*+ strain is more often found in patients with gastric adenocarcinoma than in those without the disease (21). The increased inflammation caused by more virulent strains appears to be related to some of the gene products from the *cag* pathogenicity island, a region with *cagA* as the marker sequence. Furthermore, cytotoxicity of a strain is also affected by the *vacA* genotype present, in that peptic ulceration is associated with the strain's *vacA* signal sequence (21).

Although testing for the presence of the *cagA* marker sequence is the primary viable diagnostic option predictive of disease at this time, the natural history of infection caused by strains testing negative for *cagA* is not altogether clear. Determination of virulence factors remains a research goal, at present, and has not yet been incorporated into algorithms for the management of *H. pylori* (21).

Transmission

H. pylori is a human pathogen with humans as the primary reservoir. However, a mode of transmission has yet to be determined with certainty. Most evidence points to both oral-oral and fecal-oral spread. Oral-oral spread is more likely the predominant means of transmission in developed countries, whereas the fecal-oral route probably accounts for most spread in developing countries (22). In addition, case reports exist of iatrogenic transmission via flexible endoscopes that have contacted multiple patients without proper disinfection (23). The housefly has been evaluated as a possible but unproven vector for transmission (24). Transmission of *H. pylori* in Asian populations was speculated to occur secondary to chopstick use, but this theory was rejected after further investigation (25).

Epidemiology

H. pylori infection is the world's most common human chronic bacterial infection, with a world-wide prevalence of at least 50 to 60% (26, 27). However, prevalence varies dramatically from region to region, particularly between developed countries with rates between 25 to 50%, and developing countries with rates as high as 70 to 90% (26, 28).

A general review of studies on the epidemiology of *H. pylori* infection in China determined the prevalence of infection to be 61% (29). A more specific study analyzing 65 Chinese counties found *H. pylori* seropositivity rates of 65% (range 28 - 94%) in northern counties and 57% (range 29 – 96%) in southern counties (30). One study

analyzing a specific southern China county found the prevalence of *H. pylori* infection to be 80%. Interestingly, this study found a prevalence of only 58% in Hong Kong which was comparable to a previous study (prevalence of 55%) (31, 32, 33). (Note that although the prevalence for Hong Kong was lower than southern China, it is still significantly higher than other developed countries.)

Furthermore, these rates are comparable to Chinese populations found in other areas of Asia. A study carried out in Malaysia, a country with large Chinese and Indian populations, found the prevalence in the Chinese cohort to be 49%. In contrast, the prevalence of infection among the Malay and Indian populations was 16% and 62%, respectively (34).

Risk Factors for Infection

The incidence of *H. pylori* infection among adults is low, estimated between 1% per year in developed countries and 3% per year for developing countries (22). Childhood appears to be the dominant time period for acquiring *H. pylori* (22, 26). Prevalence, however, varies considerably among children in different regions; it appears that acquisition is low in the children of developed countries, while in the developing world this age group represents the major period of new infection (22). It has been hypothesized that an 'environmental pool' of *H. pylori* exists to which children are exposed in developing countries; in developed countries this source has either been eliminated or children are not exposed to it. Either possibility is believed secondary to improvements in sanitation, refrigeration and water purification. Consequently, adults more than 50 years of age in developed countries maintain a high prevalence of infection (probably acquired

in childhood), while prevalence is low among children in developed countries. The present positive correlation between age and prevalence of infection is thought to be due to a birth cohort effect (26).

Notable correlations do exist across all ages in which indirect relationships for both socioeconomic status and education level and *H. pylori* infection have been shown; other potential risk factors such as gender and alcohol use have been investigated, but display no consistent relationship with prevalence. Interestingly, gastroenterologists and endoscopists have been shown to display increased seropositivity for *H. pylori* (22).

Treatment

Despite mounting evidence supporting *H. pylori's* role in causing peptic ulcer disease, many clinicians remained skeptical. It was not until February 1994 that the National Institutes of Health convened a Consensus Development Conference Panel which reviewed contemporary *Helicobacter pylori* research and concluded that peptic ulcer disease was often an infections disease and should be treated as such.

Active *H. pylori* infection has been shown to be effectively treated by several treatment protocols. The most successful in the U.S. consist of two weeks of acid suppression combined with at least two antibiotics. The most popular combinations use a proton pump inhibitor with either amoxicillin and a macrolide (typically clarithromycin) or with a nitroimidazole (typically metronidazole) and a macrolide (35). These treatment protocols cure *H. pylori* in 85 to 95% of patients enrolled in Western studies (36). Since the outcome of therapy is probably dependent in part on antibiotic resistance, these rates may differ in Asia due to the significantly higher levels of metronidazole resistance

present there. For all strains of *H. pylori*, levels of metronidazole resistance as high as 50% have been reported in Hong Kong and Singapore (80 to 90% levels of resistance were reported in India) (37).

In the future, therapy for *H. pylori* may consist of vaccination. Current efforts in mice have succeeded in producing vaccines that not only prevent infection, but also cure preexistent *H. pylori* infection. However, these vaccines, which use a *H. pylori* urease, cannot yet be applied to humans since they require an adjuvant detrimental to humans, such as cholera toxin. A more efficacious vaccine may result from efforts to utilize *H. pylori* outer membrane proteins (OMPs), such as porins, to produce an effective immune response (36).

Eradication

Although *H. pylori* is the most common chronic bacterial infection in humans with definite links to gastrointestinal pathology, most infected people never display clinical signs or symptoms of disease (27). Consequently, arguments have been made for and against widespread vaccination or eradication of this organism and whether eradication would be cost effective (38, 39). Some studies have even suggested a beneficial role for *H. pylori* in protecting against gastroesophageal reflux disease (GERD) (40, 41) and possibly a secondary protection from the GERD-induced esophageal cancer. Yet, in patients with PUD, eradication of *H. pylori* decreases the recurrence rate of PUD to 4%, from 80% (or 25% if on long-term histamine-2 (H_2) receptor blockers).

Diagnostic Tests for Infection

As awareness of the role of *H. pylori* in gastroduodenal diseases has increased, many patients have sought testing and treatment from their primary care physicians. Invasive biopsy-based tests, including culture, histology and the rapid urease test, are effective in diagnosing *H. pylori* infection. However, considerable investments in facilities, equipment, expertise and time are required to perform these tests. Invasive tests are not a practical option for the primary care setting in which testing is performed at the site.

Non-invasive tests are attractive for this setting in that they do not have many of these requirements. Additionally, non-invasive tests eliminate the potential for sampling error of the biopsy-based tests due to the patchy distribution of *H. pylori* in some patients. In fact, screening young dyspeptic patients for *H. pylori* by non-invasive tests such as serology has been shown to reduce the need for endoscopy by up to 30% without missing significant disease in Western countries (42, 43, 44) and economic modeling suggests this strategy is cost effective (45). Still, conventional non-invasive tests such as serum enzyme-linked immunosorbent assays (ELISA) and urea breath tests also require laboratory support which itself is not widely available in primary care settings.

Commercial rapid whole blood tests for *H. pylori* have been introduced in recent years. These tests do not require separation of serum, and results are available within minutes without the need of laboratory assistance. It is important to note that these near-patient rapid whole blood tests, unlike ELISA assays, are qualitative tests. Still, preliminary studies found these commercial kits may be as accurate as laboratory-based serology tests (46, 47, 48), when tested in Western populations.

Due to the antigenic heterogeneity of *H. pylori*, the performance of commercial serology tests varies considerably among different populations (49, 50). To date, most published data have been based on Western populations (46, 47, 48, 51, 52, 53, 54, 55, 56, 57, 58). Whether these results can be reproduced in Asians remains unknown.

Statement of Purpose and Hypothesis

The aim of this study was to assess the performance of the rapid whole blood test, FlexPack HP (Abbott Laboratories, North Chicago, Illinois), in Chinese patients with dyspepsia. It was our hypothesis that the Abbott FlexPack HP Test for IgG antibodies to *Helicobacter pylori* infection in whole blood is accurate for diagnosing *H. pylori* infection in Chinese (specifically Hong Kong Chinese) patients with clinical signs and symptoms of upper gastrointestinal tract disease.

Materials and Methods

Location of Study

This study was carried out at the Prince of Wales Hospital in Hong Kong, China. Prince of Wales Hospital, the first fully air-conditioned Government hospital in Hong Kong, was officially opened in 1984. Located in the city center of Shatin, it is an acute general hospital as well as the teaching hospital of the Medical Faculty of the Chinese University of Hong Kong. With more than 1,400 beds and a total workforce of over 4,000, it is the regional hospital of the Eastern New Territories serving the population of Shatin, Tai Po, Northern New Territories, Sai Kung and the outlying islands (59).

Protocol

Consecutive patients about to undergo esophagogastroduodenoscopy to evaluate dyspepsia were considered for enrollment between June 17, 1997 and August 6, 1997. My goal was to enlist 100 patients, a sample size selected arbitrarily. Exclusion criteria were age less than 18 years, previous treatment for *H. pylori*, previous gastric surgery, and current use of antibiotics, proton pump inhibitors, or H_2 receptor blockers. Informed verbal consent was obtained from all subjects by research nurses. All patients underwent endoscopy (Olympus XQ-200; Olympus, Tokyo, Japan) with biopsy in the Endoscopy Center at Prince of Wales Hospital, performed by the center's staff physicians. Three antral and two body biopsy samples were taken from the stomach: one antral biopsy was used for rapid urease testing and the remaining four biopsies were sent for histological evaluation. The rapid urease test (CLOtest, Delta West, Bentley, Australia) was performed at room temperature and was considered negative if no colorimetric change

occurred within 24 hours. Specimens destined for histologic inspection were placed in 10% formalin, transported to the hospital's pathology department and subsequently stained with hematoxylin and eosin. The hospital's staff pathologists determined histological diagnosis.

All patients also underwent [^{13}C] urea breath testing (UBT) at least two hours after endoscopy. UBT's were performed either by a research nurse or by myself; the laboratory analyzed all results. Following a previously described method (60) the fasting patient first drank a test meal consisting of a 200 ml concentrated citrate solution to delay gastric emptying. Following a two-minute delay, three baseline breath samples were collected. Samples were obtained by having the patient blow through a straw resting inside of a 10ml test tube. The straw was slowly withdrawn from the tube over approximately a four-second period until a blush of condensation appeared, immediately after which the tube was capped. After an eight-minute delay, the patient swallowed a 100mg [^{13}C] urea tablet (Cambridge Isotope Laboratories Inc., Andover, Massachusetts) dissolved in 25ml of water, followed by a 25ml drink of water alone. The patient then performed one minute of exercise including deep waist bends to encourage sample mixing. After 30 minutes, three additional breath samples were obtained from the patients in the previously described fashion. All samples were covered with Parafilm until analyzed via mass spectrometry (Europa scientific 20:20 Isotope Ratio Mass Spectrometer with a RoboPrep G Automatic Breath Sampler). The $^{13}C/^{12}C$ ratio of the sample was calculated by the computer against the known isotopic ratio of the reference gas. The $^{13}C/^{12}C$ isotopic ratios were reported in the per ml format: delta ^{13}C/ml = (((($^{13}C/^{12}C$ sample)-1) X 1000)/($^{13}C/^{12}C$ Standard). Excess ^{13}C was calculated as the

difference between the baseline delta ^{13}C and 30 minute delta ^{13}C (30-minute delta ^{13}C – baseline ^{13}C). Values greater than or equal to 3.5 were considered to be positive. Patients with concordant histology and CLOtest results, but a discordant UBT result returned for a second UBT which was used as the final UBT result in all cases.

Patients were tested for *H. pylori* on the day of their endoscopy using the FlexPack HP rapid whole blood serology test kit (manufactured by SmithKline Diagnostics, Inc. for Abbott Laboratories, Abbott Park, Illinois). I performed all FlexPack HP tests. The FlexPack HP test incorporates high molecular weight cell-associated proteins as the antigen to detect *H. pylori*-specific IgG antibodies in whole blood. FlexPack HP test cards ready for study were kept at room temperature (no more than 30 days), opened within minutes of use and were prepared according to the manufacturer's specifications. After venipuncture, blood was immediately transferred from the syringe into EDTA and soybean extract-coated mini-pipets included in the test kit and directly applied to the appropriate chromatography column on the prepared test card. Following the flow of the sample through this initial column to its termination point, the test card was closed and the time was noted. After exactly four minutes the results were read in the card's test window. Within the test window the appearance of two distinct lines was treated as positive, a single control line represented a negative result, and the absence of any line indicated an invalid test. Any trace of a line in the expected positive position was regarded as a positive result per product instructions. I read all the rapid whole blood test results without knowledge of the endoscopy findings, or results from other *H. pylori* tests.

Data Analysis

The "gold standard" used to determine *H. pylori* infection was defined prior to analysis as positive results of at least two of the three reference tests performed to diagnose *H. pylori* infection (histology, CLOtest and UBT). I calculated sensitivity, specificity and positive and negative predictive values for the FlexPack HP with 95% confidence intervals using formulas created within Microsoft Excel 97 (Microsoft, Redmond, Washington) and using the Java based internet site "2-way Contingency Table Analysis," (61). Unpaired *t*-tests (mean comparison) and *Z*-tests (proportion comparison) were performed using the internet sites "*t* test: Independent Groups,"(62) and "WebStat-Proportions (p statistics): Two sample," respectively (63). A *P* value of 0.05 was considered statistically significant. Data are summarized as mean +/- standard deviation.

Results

Patient Characteristics

Characteristics of 100 patients from Hong Kong undergoing upper GI tract endoscopy for dyspepsia are displayed in Table 1. One hundred patients (55 female, 45 male) were studied. The patients' mean age was 51 +/- 15 years (range, 21 to 90 years). Fifty-eight patients (58%) were older than 45 years of age. Seventeen (17%) patients had a history of peptic ulcer disease. At esophagogastroduodenoscopy, five (5%) patients had gastric ulcers, six (6%) had duodenal ulcers, seven (7%) had gastric erosions and 20 (20%) had gastric erythema. In the remaining 62 patients, no endoscopically apparent disease was found. No patients had duodenitis or duodenal erosions.

Characteristics of *H. pylori* prevalence in the 100 patients studied are given in Table 2. Fifty-four (54%) patients were confirmed to be *H. pylori* positive based on at least two positive results among the reference tests (rapid urease test, histology, and [^{13}C] urea breath test). The *H. pylori*-positive group had a mean age (51 +/- 15 years) similar to the *H. pylori*-negative group (52 +/- 16). There was no statistically significant difference in prevalence of infection between men and women (55% vs. 53%, respectively [P = NS]).

The prevalence of *H. pylori* was highest among the six patients with duodenal ulcers and the five patients with gastric ulcers (83% and 80% respectively). The prevalence among the 20 patients with gastric erythema was 60%. Thirty of the 62 patients with no apparent disease (49%) were *H. pylori* positive and three of seven patients (43%) with gastric erosions were positive for *H. pylori*. Note that the prevalence of *H. pylori* in the group with ulcers, nine of eleven patients (82%), was significantly

different from the prevalence in the group without ulcers, 45 of 89 patients (51%) (P = 0.05).

Diagnosis of *H. pylori* Infection - Reference Panel Analysis

The relationship between reference test and FlexPack HP test results in each of the 100 patients are presented in Table 3. Concordance of all test results (including the FlexPack HP) occurred in 74 of the 100 patients. Reference tests alone were concordant in 89 of the 100 patients; in 11 patients, reference tests were discordant.

Each reference test method was discordantly positive or negative in at least one patient. Three of the 54 "*H. pylori*-positive" patients tested negative for infection by histology, two patients by UBT and one patient by CLOtest. Three of the 46 "*H. pylori*-negative" patients tested positive for infection by UBT, and two by histology.

Diagnosis of *H. pylori* Infection – FlexPack HP Test Performance

The sensitivity, specificity, positive and negative predictive values for the FlexPack HP are 82%, 85%, 86% and 80% respectively (see Table 4). Also shown on Table 4 are values for FlexPack HP's performance in the age groups less than or equal to 45, greater than 45, less than or equal to 65 and greater than 65 years of age; no statistically significant differences occurred in any of the characteristics between the age groups. Poor readability, manifested as faintly positive results, occurred in 15 FlexPack HP observations (15%). All tests were valid based on performance of built-in positive controls. However, one of the 51 positive FlexPack HP observations and three of the 49

negative observations displayed faint positive control lines. None of the tests with faint positive control lines displayed discordant results from the defined gold standard.

Among the nine patients with *H. pylori*-associated peptic ulcers, false-negative serology was encountered in two of the four (50%) patients with gastric ulceration. No false-negative FlexPack HP results occurred in the five patients with *H. pylori*-associated duodenal ulceration or in the three patients with *H. pylori*-associated gastric erosion.

Discussion

In 1994, the National Institutes of Health Consensus Development Panel on *H. pylori* recommended eradication of *H. pylori* infection in all infected patients with active peptic ulcer disease or with a history of PUD and on maintenance acid suppression. Tests for diagnosing *Helicobacter pylori* infection which are simple, accurate and rapid are needed in the near-patient setting to facilitate the diagnosis and treatment of *H. pylori*. In fact, the American Gastroenterological Association's 1998 medical position statement for evaluation of dyspepsia specifically recommended that "a locally validated noninvasive *H. pylori* test (e.g., serology or urea breath test) is undertaken to determine if the patient is infected" for patients under 46 years of age without alarm features or previous investigations (64). A European *H. pylori* study group developed similar recommendations (65).

Guidelines for the Asian Pacific region, following the initiatives of the West, were formulated in 1997. Unique characteristics of this population include the high background prevalence of infection, high rates of gastric cancer, and limited resources in the medical community. Non-endoscopic methods were deemed acceptable for this region as diagnostic tests for *H. pylori* infection, including the UBT or a locally validated antibody test (66). Clinicians in Hong Kong, being members of this region, played a major role in formulating these guidelines.

In previous studies, prevalence of *H. pylori* infection in Hong Kong was 55 – 58%, a rate comparable to the value of 54% found in the present analysis. Although Hong Kong is a developed country, its population displays a high *H. pylori* prevalence, even in younger age groups. For example, the 21 to 30 year age group in previous studies

displayed a prevalence of 53%, while our study determined a value of 52% for those no older than 45 years of age (31, 32, 33). (The 21 to 30 year age group in our study consisted of only eight members, however, 50% were positive for *H. pylori* infection.)

The analysis of *H. pylori* prevalence in relation to endoscopic diagnoses in this study is also similar to previous studies. While the number of cases are low in each diagnostic category (see Table 1), values of 48%, 80% and 83% prevalence were found for no apparent disease, gastric ulcer and duodenal ulcer patients, respectively. These values compare to those from native Southern Chinese patients of 45%, 76% and 75%, respectively. In studies highlighting Hong Kong Chinese, duodenal ulcer was noted to be positive for *H. pylori* in excess of 90% (67).

Considering the extent of the problem of *H. pylori* infection in the Hong Kong Chinese population, a comprehensive plan was required to diagnose and treat positive cases. As noted earlier, an antibody test was deemed acceptable for this population if locally validated. Most serology studies prior to this, however, were carried out in Western populations and few had previously assessed whole blood serology tests. Subsequent studies have shown commercial rapid whole blood serology tests to be adequately accurate and potentially useful for in-office *H. pylori* testing if clinicians are aware of the limitations of the tests (68, 69). Clinicians must remember that these tests can only indicate the presence of specific IgG antibodies to *H. pylori*, but do not distinguish between current and past *H. pylori* infection; furthermore, a negative test may indicate that *H. pylori* antibodies either are not present or are present at levels too low for detection, as may be the case early in infection. Although these near-patient, rapid whole

blood tests appeared appropriate for the needs of the Hong Kong Chinese population, they required local validation.

This is the first analysis of a rapid whole blood test for *H. pylori* in the Hong Kong Chinese population. In this study, the overall performance of the FlexPack HP test in Hong Kong Chinese patients was comparable to Western results for other commercial serology kits. Our study determined a sensitivity of 82% (CI 73 – 88%) and a specificity of 85% (CI 74 – 92%) for the FlexPack HP which compares favorably to sensitivities of 83 to 96% and specificities of 70 to 93% from the West (46, 47, 48, 51, 52, 53, 54, 55, 56, 57, 58).

Previously, discrepancies between Western and Asian populations in the performance of commercial serology tests for *H. pylori* were reported (49, 58, 70). A study from Thailand showed that a commercial ELISA (Pylori Stat; BioWhittaker, Walkerville, Maryland) was inferior to an ELISA developed from local *H. pylori* strains (49). Prior experience at Prince of Wales Hospital with commercial ELISAs in a group of Chinese patients was also disappointing (70). The performance of another rapid whole blood serology test, the Helisal One-Step, was recently evaluated between two groups of European and South Asian patients in a study from Britain (58). The authors reported unexpectedly poor results in Asian patients (sensitivity, 79 to 81% versus 93 to 96%; specificity, 42 to 50% versus 57 to 64%).

The reasons for these earlier discrepant results are unclear. Poor sensitivity in prior comparisons is possible if antibody is absent, in a concentration too low to detect, or non-reactive to the antigen in the assay. In earlier studies, poor sensitivity may be accounted for by the considerable antigenic heterogeneity of *H. pylori*. By using bacterial

isolates and sera from different continents, Hook-Nikanne *et al.* demonstrated that antigens prepared from individual bacterial strains obtained from North America and China were not sensitive enough for serological detection of *H. pylori* in a heterogeneous population (50). This phenomenon may be overcome by using pools of bacterial strains obtained from different ethnic groups. If the patented antigen utilized in the FlexPack HP, high molecular weight cell-associated proteins (HM-CAP™), is of the pooled type, it could explain the comparable sensitivity realized in this study.

Our study also demonstrated specificity for FlexPack HP comparable to Western analyses. Poor specificity in previous comparisons may be accounted for by the high carriage of other cross-reacting intestinal pathogens in developing countries, such as *Campylobacter* species, which may produce false-positive serological results (46). This problem is less likely with FlexPack HP since it has been previously shown to have no cross reactivity with a number of bacteria, including *Campylobacter jejuni*, *Campylobacter fetus*, *Campylobacter coli*, *Escherichia coli*, and *Helicobacter mustalae* (71). An alternate explanation involves the inadvertent use of antibiotics for respiratory and intestinal infections in the community which may inhibit or even eradicate *H. pylori*. Since antibody can persist in serum for months after eradication, serological results may be falsely-positive after eradication and contribute to decreased test specificity. Previous studies may not have controlled for this possibility. The present study excluded patients previously treated for *H. pylori* or currently using antibiotics.

In North America and Europe, the test-and-treat strategy has been adopted for the management of dyspepsia (64, 65). Serology, being more widely accessible than the urea breath test, is likely to become more popular for this purpose. Our study found overall

sensitivity and specificity to be similar to that for series carried out in the West. A study in a larger population of Hong Kong Chinese less than 45 years of age may be appropriate before this serology kit is used in test-and-treat protocols in this age group. Furthermore, our analysis found that 50% of *H. pylori*-associated gastric ulcers (two of four gastric ulcer patients, or two of nine [22%] peptic ulcer patients overall) would have been missed had endoscopy been withheld in these patients with negative rapid whole blood serology tests. Lastly, the risk of missing young patients with gastric cancer has not yet been considered.

In conclusion, the FlexPack HP is quick and convenient to use and performed adequately in a secondary care setting in Hong Kong Chinese patients. This test may be useful for in-office *H. pylori* testing if clinicians are aware of the limitations of the test.

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Table 1. Characteristics of 100 Consecutive Patients From Hong Kong Who Had Esophagogastroduodenoscopy to Evaluate Dyspepsia

Age	51 +/- 15 years [a]
Gender	
Female	55%
Male	45%
Prior ulcer disease	17%
Endoscopic diagnosis	
No apparent disease	62%
Gastric erythema	20%
Gastric erosion	7%
Duodenal ulcer	6%
Gastric Ulcer	5%

[a] Mean +/- standard deviation

Table 2. Characteristics of *H. pylori* Prevalence in 100 Patients From Hong Kong Who Had Esophagogastroduodenoscopy to Evaluate Dyspepsia [a]

Age	
H. pylori-positive group (n = 54)	51 +/- 15 years [b]
H. pylori-negative group (n = 46)	52 +/- 16 years
Overall *H. pylori* prevalence	54%
H. pylori prevalence for endoscopic diagnosis	
Duodenal ulcer (n = 5)	83%
Gastric ulcer (n = 4)	80%
Gastric erythema (n = 12)	60%
Gastric erosion (n = 3)	43%
No apparent disease (n = 30)	48%

[a] Patients were confirmed to be *H. pylori* positive based on at least two positive results among the reference tests: rapid urease test, histology, and [13C] urea breath test.

[b] Mean +/- standard deviation

Table 3. Relationship Between Reference Test Results and FlexPack HP Results Among 100 Patients With Dyspepsia From Hong Kong [a]

	Number of Reference Tests Positive			
	0	1	2	3
FlexPack HP Positive	6	1	5	39
FlexPack HP Negative	35	4	1	9
Total	41	5	6	48

[a] Patients were confirmed to be *H. pylori* positive based on at least two positive results among the reference tests (rapid urease test, histology, and [^{13}C] urea breath test).

Table 4. Performance Characteristics of FlexPack HP, a Rapid Whole Blood Serology Test For *H. pylori* Infection, Among 100 Patients With Dyspepsia From Hong Kong

Group	Number	Sensitivity (% [CI]) [a]	Specificity (% [CI])	Predictive value (% [CI])	
				Positive	Negative
Overall	100	82 (73 - 88)	85 (74 - 92)	86 (77 - 93)	80 (70 - 86)
<= 45 years	42	73 (57 - 82)	85 (67 - 96)	84 (66 - 95)	74 (59 - 83)
> 45 years	58	88 (76 - 95)	85 (70 - 93)	88 (76 - 95)	85 (70 - 93)
<= 65 years	82	80 (71 - 87)	83 (71 - 92)	86 (76 - 93)	77 (65 - 85)
> 65 years	18	88 (56 - 99)	90 (65 - 99)	88 (56 - 99)	90 (65 - 99)

[a] CI = confidence interval.

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